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Influence of Minimal Residual Disease at Day 15 of Induction Therapy on Survival of Children with Acute Lymphoblastic Leukemia

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Abstract

Objective. The aim of the study was to evaluate the impact of minimal residual disease (MRD) on day 15 of induction therapy (d15) on the treatment outcome in children with acute lymphoblastic leukemia (ALL). **Materials and Methods.** The study included 74 patients (1-18 years) with ALL, who were treated at the Pediatric Clinic of the University Clinical Center Banja Luka from January 2011 to May 2021. All patients were treated according to ALL IC-BFM 2009 protocol. MRD on bone marrow was assessed d15, using the multiparameter flow cytometry method (FCM). **Results.** Of all, 59.46% of patients had MRD d15 0.1−10%, MRD<0.1% had 18.92% of patients, and 21.62% had MRD >10%. Patients with the lowest MRD had the highest 5-year overall survival (OS) and event-free survival (89.5% and 91% respectively) and the lowest cumulative risk for relapse or death (9.7% and 8.1%), in contrast to patients with MRD>10% in whom OS was 80.0%, and the risk of recurrence is 20%. Predicted MRD d15 was significantly associated with prednisone response assessed in the peripheral blood on day 8 (P<0.001) and statistically significantly positive correlation (r=0.498; P<0.001) was found. **Conclusion.** MRD measurement d15 has a great prognostic significance for patients in the standard and high risk groups, but not for patients in the intermediate risk group. The introduction of additional testing is necessary for better identification of patients with an increased risk of disease recurrence.

Key Words: Minimal Residual Disease ▪ Acute Lymphoblastic Leukemia ▪ Children ▪ Outcome.

Introduction

Modern treatment of childhood acute lymphoblastic leukemia (ALL) is based on the individualization of therapy according to risk groups, which implies intensification of therapy for high-risk groups and optimization of therapy for low-risk groups, with the aim of achieving long-term remission while minimizing toxic complications of chemotherapy (1, 2). Thanks to risk-adapted therapy approach and better supportive care, the 5-year survival of children with ALL has increased significantly over the last decades, from 50% to 92% (3, 4). However, disease relapses are still the main cause of poor treatment outcome and occur in about 20% of children with ALL (5, 6).

Furthermore, it has been proven that the final outcome is influenced by many biological and clinical characteristics such as the age of the child, the number of leukocytes and infiltration of the central nervous system at diagnosis, cytogenetic characteristics of the disease as well as the initial response to therapy (1). Therefore, identifying the most sensitive prognostic factors for predicting relapse was of great importance in order to realize the concept of "risk-adjusted therapy" (6-10). It has been shown that an early treatment response is a significant prognostic indicator and a predictive factor for disease recurrence (9, 10). Patients with good prednisone on day 8 with a significant reduction in the number of blasts in the peripheral blood as well as a reduction in blasts in the bone marrow on day 15 have a significantly better prognosis (10). However, rapid development and modeling of therapy intensity have shown that their

association with relapse is not perfectly correlated. Some of the reasons are great morphological similarity of all blast cells with bone marrow lymphoid precursors (hematogones) and often with mature lymphocytes. Compared to standard bone marrow cytomorphology, minimal residual disease (MRD) analysis enables the detection of one blast cell per 10,000 to 100,000 normal cells, which represents a 100-fold higher sensitivity compared to standard cytomorphological analysis (11).

Today, two methods are most commonly used for the assessment of MRD, flow cytometry (FC) for the analysis of aberrant immunophenotypes and polymerase chain reaction (PCR) method with amplification of various fusion gene receptors for immunoglobulins or T-cell gene receptor rearrangements. Assessment of MRD during the treatment of various hematological malignancies has a high predictive value for disease recurrence, and therefore worse event free survival (ES) as well as overall survival (OS) (4, 11–13). Personalization of therapy based on MRD thus may improve the treatment outcome of children with ALL (4). Therefore, FC-MRD monitoring in different time points is used in current protocols and is the main criterion of stratification therapy with a more precise selection of intensity and duration (3, 14–18).

The aim of the study was to evaluate the impact of FC-MRD measured on day 15 of induction therapy (d15) on the treatment outcome in children with ALL.

Material and Methods

Patients and Treatment Protocol

The retrospective study included 74 patients aged 1 to 18 years, with newly diagnosed ALL, who were treated at the Pediatric Clinic of the University Clinical Center Banja Luka from January 2011 to May 2021. The Ethical Committee of University Clinical Centre Banja Luka approved the study (No. 01-19-360-2/23). All children were treated according to ALL IC-BFM 2009 protocol and were divided into three risk groups: standard, intermediate and high risk. Stratification of patients into risk groups was defined according to the treatment protocol, based on clinical, laboratory and genetic characteristics of the disease as well as initial response to therapy - prednisone response d8 and MRD d15 of therapy (19). The measurement of the absolute blast count (ABC) in the peripheral blood on d8 (after 7 days of prednisone and one intrathecal therapy) in the induction phase was done in all patients. Whereas, ABC lower than 1000/μL is considered as a good prednisone response (PGR), and ABC 1000/μL and higher as a poor prednisone response (PPR). In addition, MRD was assessed in all patients in a bone marrow sample obtained by bone marrow aspiration from the iliac crest after 14 days of corticosteroid therapy (prednisone), single doses of daunorubicin and vincristine and asparaginase, and two intrathecal therapies with methotrexate.

Sample Preparation and Multiparameter Flow Cytometry

In our study, MRD assessment was conducted by the eight-color FC (BD FACSCanto II) at the Department of Clinical Pathology and Immunophenotyping of the Institute for Health Care of Mothers and Children of Serbia "Dr. Vukan Čupić", Belgrade. The standardization process, including sample preparation, monoclonal antibody clone selection, staining-lysis procedure, and flow cytometric analysis, was performed according ALL IC-BFM 2009 protocol.

Briefly, bone marrow samples were transported immediately after aspiration in an ethylenediamine tetra acetic acid tube and analyzed by FC within 24 hours, using standard whole bone marrow lysis protocols (20, 21). Per sample, a standard acquisition consisted of 3×10^5 cell events. For FC analysis, samples were incubated with fluorochromes conjugated to antibodies specific for the proteins of interest. A stream of individual cells passes through multiple lasers that excite each fluorochrome and the emitted fluorescence intensity is captured and converted into digital signals that can be analyzed. Until 2019, a panel of four color combinations was used, and since then

an eight-color panel. For B-cell precursor ALL (B ALL) it was CD58/CD10/CD34/CD19/CD20/CD38/SYTO41/CD45, D10/CD11a/CD34/CD19/CD20/CD38/SYTO41/CD45 and for T-cell ALL (T ALL) CD99/CD56/ CD3/CD5/ iCD3/CD7/SYTO41/CD45 (antibodies ordered by channel sequence: fluorescein isothiocyanate, phycoerythrin, peridinin-chlorophyll-protein, allophycocyanin, phycoerythrin-cyanin 7, allophycocyanin-cyanine 7, violet 450, Violet 500. Data collection and analysis was performed using Diva software (BD Life Sciences, San Jose, CA USA).

Addionally, live cell permeant SYTO 16 or SYTO 41 nucleic acid fluorochrome staining (Invitrogen™, Thermo Fisher Scientific, Waltham, MA USA) was used to exclude residual anucleated erythroid cells, platelets or debris. This staining allowed unbiased proportional quantification of MRD among total NC (SYTO+).

Overall Survival and Event-Free Survival Definition

The OS was defined in months from disease diagnosis to death or last contact, and EFS from disease diagnosis to some event: relapse, death or follow-up contact. All data related to patient demographic characteristics (age, sex), immunophenotypic, cytogenetic, and molecular characteristics of leukemia, early response to prednisone, and FC-MRD assessment of were collected from the hospital's electronic database and patient medical history.

Statistical Analysis

The difference in the frequency of the observed characteristics according to patient groups was evaluated by Pearson's χ^2 contingency test. The OS and EFS survival were estimated by the Kaplan-Meier method, and the significance of differences between different risk groups in terms of OS and EFS survival was tested by the log rank test. The cumulative risk of recurrence or death is shown as a Hazard Ratio (HR). Spearman's non-parametric correlation was used to determine the degree of association (correlation) of the characteristics. The SPSS program for Windows (SPSS, Chicago, IL, USA) was also used for statistical analysis, whereas $P<0.05$ was considered significant.

Results

Thirty-nine boys (52.7%) and 35 girls (47.3%) aged 1 to 18 years, with an average age 6.88 years (median 4.0 years) were included in the study. The mean follow-up time was 78.5 months (from 36 to 120 months). Precursor B ALL was diagnosed in 59 patients (79.73%), and T ALL in 15 patients (20.27%). Considering the risk group, patients were stratified into three groups: the intermediate risk group, high risk group and standard risk group (Table 1).

Regarding FC-MRD d15, 44 patients (59.46%) had MRD 0.1–10%, 14 (18.92%) <0.1%, and 16 (21.62%) patients >10%. Sixty-four (86.49%) patients had GPR d8, 64 (86.49%). The main event

Table 1. Demographic and Clinical Characteristics of Patients

Characteristics	Value
Age: Median (IQR*)	4.0 (3.0–12.0 yrs)
Gender: Male (N; %)	39 (52.70)
Imunofenotype (N; %)	
T cell	59 (79.73)
B cell	15 (20.2)
MRD† (N; %)	
<0.1%	14 (18.92)
0.1–10%	44 (59.4)
>10%	16 (21.62)
RISK group (N; %)	
Standard risk	7 (9.40)
Intermediate risk	48 (64.90)
High risk	19 (2570)
Cytogentics (N; %)	
Normal	20 (27.03)
Hyperdiploidia	19 (25.68)
Hypodiploidia	4 (5.40)
No results	31 (41.89)

*Interquartile interval; †Minimal residual disease.

Table 2. Distribution of Events According to FC-MRD at 15 Day of Induction Therapy

Events	FC-MRD*			
	<0.1%	0.1-10%	>10%	Overall
	N (%)			
Overall	14 (18.92)	44 (59.46)	16 (21.62)	74 (100)
Relapse	1 (10.00)	8 (80.00)	1 (10.00)	10 (12.16)
Death	1 (10.00)	6 (60.00)	3 (30.00)	10 (12.16)
After relapse	1	6	-	7 (70.00)
In induction	-	-	3	3 (30.00)

*Flow Cytometry-Minimal Residual Disease.

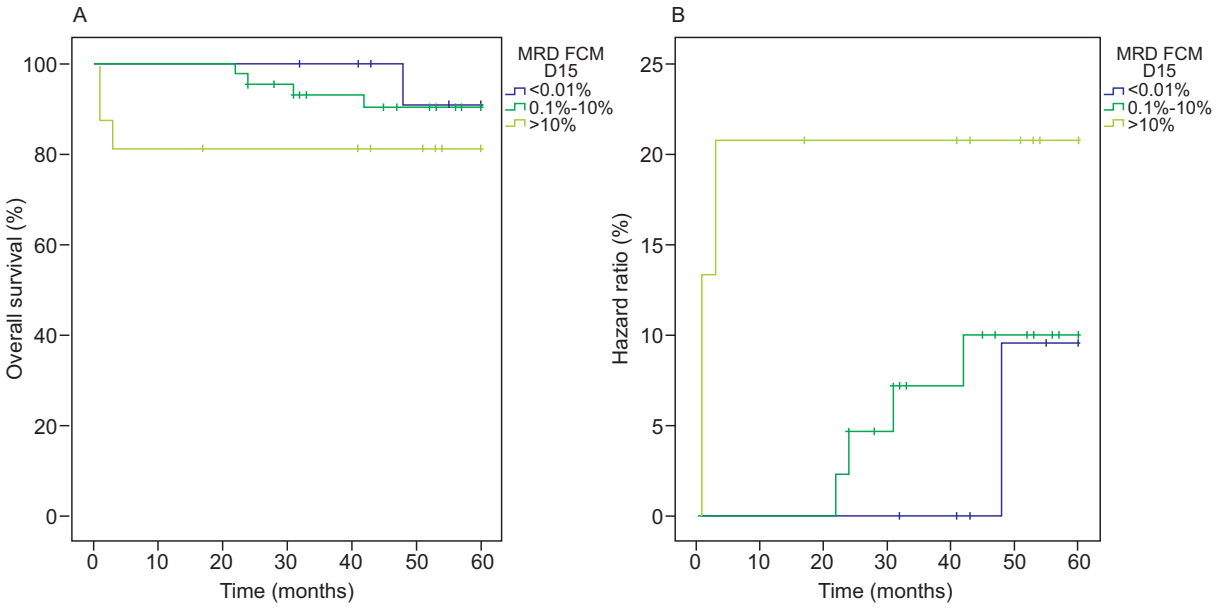

Figure 1. (A) Estimated overall survival according to FC-MRD at day 15 of induction therapy by flow cytometry and (B) cumulative hazard ratio for death according to MRD at day 15. D=Day; MRD=Minimal residual disease.

that affected the survival of our patients was disease recurrence: 10 (12.16%) patients had a recurrence. We found that the majority (8 patients, 80%) relapsed from group with MRD d15 0.1-10%, and 1 patient each with MRD d15 <0.1% and >10% (Table 2).

Ten (12.16%) patients died, of which disease recurrence was the cause of death in 7 (70%) patients. The other three patients died in induction phase and had high MRD d15 (above 10%), one patient died from an anaphylactic reaction to asparaginase, two from severe infection, and none of these three patients achieved remission d33.

The 5-year OS of patients with MRD d15 <0.1%, 0.1–10% and >10% was 89.5%, 88% and 80.0%, respectively (Figure 1A). The cumulative hazard ratio (HR) for death or relapse was 9.7%, 10.2% and 20%, respectively (Figure 1B) confirming that patients with FC-MRD d15 <0.1% had the highest OS with the lowest risk for relapse or death and that patients with MRD >10% had the lowest OS and the highest risk of recurrence or death. The log rang test determined that differences in OS according to MRD were not statistically significant (P=0.384).

Similar results were obtained in the analysis of the 5-year EFS. The highest EFS was in patients with MRD d15 <0.1% (91%), and the lowest EFS was in patients with MRD d15 >10% (72%) (Figure 2A). Also, the lowest risk for recurrence

Table 3. Distribution of FC-MRD at 15 Day of Induction Therapy according to Prednisone Response

FC-MRD*	Good prednisone response N (%)	Poor prednisone response N (%)	P-value†
< 0.1%	17 (26.56)	0 (0)	
0,1–10%	40 (62.50)	1 (10)	<0.001
>10%	7 (10.94)	9 (90)	
Total	64 (100)	10 (100)	-

*Flow Cytometry-Minimal Residual Disease: †Pearson ^{2} test.

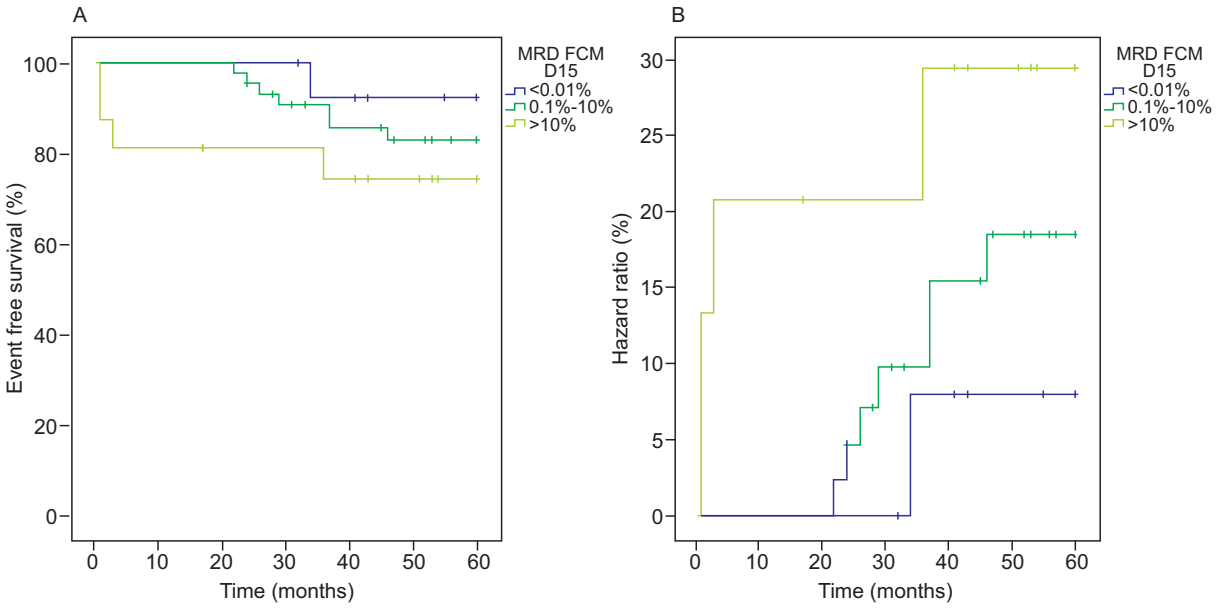

Figure 2. (A) Estimated event-free survival according to FC-MRD at day 15 of induction therapy by flow cytometry and (B) cumulative hazard ratio for relapse according to MRD at day15. D=Day; MRD=Minimal residual disease.

or death was in patients with MRD d15 <0.1% (8.1%), and the highest in patients with MRD d15 >10% (29.9%) (Figure 2B). The log rang test determined that differences in EFS according to MRD were not statistically significant (P=0.341). From patients with PPR, 90% had FC-MRD d15 >10%, and only one patient 0.1% to 10%. Whereas, no patient with MRD d15 <0.1% had PPR (P<0.001) (Table 3). Additionally, when bone marrow FC-MRD d15 and peripheral blood ABC d8 values were compared, a statistically significant positive correlation was found (r=0.498; P<0.001).

Discussion

Demographic and clinic characteristics of study patients are in agreement with the results of other studies (1, 22–25). By modulating the intensity of therapy based on the precise stratification of patients depending on the risk of disease recurrence, significant progress has been achieved in the treatment of pediatric ALL, with a five-year survival rate of around 80% in developed countries (2, 3). Assessment of early response to therapy plays a significant role in the treatment and cure of children with ALL (4, 13–16). Furthermore, long-term experience has shown that the minimization of leukemic cells in the peripheral blood on d8 of therapy, known as the prednisone response, and in the bone marrow (morphological and FC-MRD assessment) represents an important factor in assessing the success of therapy and predicting the disease recurrence, and therefore has become an integral part of the BFM protocol and criteria for stratification of patients into risk groups (13). Additionally, in the modern therapeutic protocols

such as ALL IC-BFM 2009, MRD assessment at different time points is used to assess therapeutic response and optimization of further treatment (18, 20, 26–28).

In the previous treatment protocol that we also used, ALL IC-BFM 2002, only bone marrow cytomorphology day 15 and 33 was used to assess the therapeutic response. When the results of morphological remission were compared with FC-MRD values in bone marrow day 15 and 33, a significant correlation was observed. However, it was determined that this positive correlation applies more to patients who are stratified into the high risk group, and less to the low risk group (20, 27). Since the main goal of the treatment strategy for children with ALL is the reduction of all toxicities, it is also important to identify low-risk group patients who require less intensive therapy (4). Nevertheless, the assessment of therapeutic response by morphological analysis of bone marrow is still of great importance and is used as the only assessment in countries with limited resources (20). By analyzing MRD d15, our results showed that patients with MRD <0.1% had a much better outcome (OS 89.5%, EFS 91%), compared to patients with MRD d15 >10% (80%, 72%). The same results apply to the overall cumulative risk of relapse or death, which is highest in patients with MRD d15 >10% (20%, 29.9%), and lowest in patients with MRD <0.01% (9.7%, 8.1%). Although our results were not statistically significant, they are consistent with the results of other similarly designed studies and confirm the importance of early MRD measurement in predicting prognosis (13, 29).

However, in contrast with AIEOP-BFM-2000 study, we cannot state that the MRD d15 is the most important predictive prognostic, which especially applies to patients in the intermediate risk group (18, 20). The main event that affected the survival of our patients was the recurrence of the disease, which occurred in 12.16% of patients, which was related predominantly to patients, with MRD d15 0.1–10%, (80% patients). Recurrence was also the most common cause of death in our patients (70%). Similar results were obtained by other authors with a significantly higher incidence of relapse and death in patients in the intermediate risk group, which confirms the fact that MRD d15 assessment cannot clearly define all patients with a high risk of disease recurrence (30, 31). In our study disease relapse was also recorded in one patient with the lowest MRD d15 values, suggesting that genotypic and phenotypic heterogeneity of leukemia, in addition to MRD, significantly influence the outcome of the disease (8). Also, despite the clear standardization of tests for MRD d15 assessment, other testing limitations, the possibility of diagnostic errors and the sensitivity of the tests should not be ignored (29). Early assessment of prednisone response as well as bone marrow morphological and FC-MRD d15 analysis are an integral part of all BFM protocols and stratifying of patients into risk groups. The results of our study showed that no patient with FC-MRD d15 <0.1% had PPR, and that 90% of patients with PPR had FC-MRD d15 >10% (P<0.001), which is consistent with the results of other studies (20, 26, 27). Our study also found a statistically significant positive correlation between prednisone response d8 and MRD d15 (r=0.498; P<0.001). Data from the literature have shown that prednisone response and morphologic criteria can identify most patients with MRD d15 >10%, but such a correlation does not apply to patients with a lower MRD values, who represent the target group for reducing the intensity of therapy and the absence of long-term complications (4, 27).

Limitations of the Study

Despite that our study has certain limitations such as retrospective nature of data analyses, relatively small number of patients which was influenced by fact that this was single center experience we summarized that our results are very important as this was first publication about children ALL in our country.

Conclusion

Our study is the first study in Bosnia and Herzegovina that showed the impact of bone

marrow FC-MRD d15 on the classification of patients into risk groups and the disease. The results confirmed the importance of MRD d15 for patients in the low and high-risk groups, but not for patients in the intermediate risk group. Given the large proportion of patients with a poor outcome from this risk group, in order to better identify patients with an increased risk of relapse, additional testing of MRD on day 33, is necessary in our center.

What Is Already Known on This Topic:

The identifying of most sensitive prognostic factors for predicting disease relapse was of great importance for the concept of "risk-adjusted therapy". It was shown that an early treatment response is a significant prognostic indicator and predictive factor for disease recurrence. MRD assessment during the treatment of various hematological malignancies has a high predictive value for disease recurrence, and therefore EFS and OS. Personalization of therapy based on MRD thus may improve the treatment outcome of children with ALL. Therefore, the monitoring of MRD levels in different time points is used in current protocols and is the main criterion of risk group assignment and risk-adjusted therapy.

What This Study Adds:

Our study is the first study in Bosnia and Herzegovina that showed the impact of bone marrow FC-MRD d15 on the stratification of patients into risk groups and the outcome of the disease. The results of our research confirmed the importance of MRD measurement d15 for patients in the low and high risk groups, but not for patients in the intermediate risk group. In order to better identify patients with an increased risk for relapse, it is necessary to implement additional MRD testing on day 33 of the therapy.

Author's Contributions: Conception and design: JSP; Acquisition, analysis and interpretation of data: BĐB and DMZ; Drafting the article: BĐB and DMZ; Revising it critically for important intellectual concept: JSP; Approved final version of the manuscript: JSP.

Conflict of Interest: The authors declare that they have no conflict of interest.

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